Amendments to the Specification:

Please replace the current Table 2 with the replacement Table 2 attached hereto as **Exhibit 1**.

Please replace the current Abstract of the Disclosure with the replacement Abstract of the Disclosure attached hereto as **Exhibit 2**.

Please amend the first paragraph on page 1 as follows:

This application is a <u>divisional of</u> ~~continuation of co-pending~~ U.S. Application No. 09/175,828, filed October 20, 1998, now U.S. Patent No. 6,221,643, which is a continuation of U.S. Application No. 08/559,303, filed November 15, 1995, now U.S. Patent No. 5,824,501, the contents of which are hereby incorporated by reference in their entireties.

Please amend the paragraph on page 3, line 29 through page 4, line 18 as follows. Note that the underlining of the word "supra" appears in the original text.

Figure 1A represents the genetic map of the *BLM* region of 15q. On the upper horizontal line, the order and distances (shown in kilobase "kb") between the polymorphic microsatellite loci were estimated by long-range-restriction mapping (Straughen, J., et al. Physical mapping of the <u>bloom syndrome</u> region ~~containing the Bloom's syndrome gene~~ BLM by the identification of YAC and P1 clones from human chromosome 15 <u>band q26.1</u>. Genomics <u>35(1): 118-128, 1996</u> ~~, 1995, submitted~~). The distance between *D15S127* and *FES* (not indicated) was determined to be 30 kb by restriction enzyme mapping of a cosmid contig (see below). Vertical lines indicate the position of the marker loci, and the circle represents the centromere. The interval between loci *D15S1108* and *D15S127* is expanded below the map. Vertical lines intersecting mark the unmethylated CpG-rich

regions identified by long-range restriction mapping, and arrows indicate the direction of

transcription of three genes in the region. Certain YACS, Pls, and cosmids (Y, P, and c,

respectively) from the contig (Straughen, et al., supra) are depicted by horizontal lines

underneath the map. Dashes on the YAC lines indicate internal deletions. At the top of the

figure, the horizontal cross-hatched bars indicate regions proximal to BLM that remained

heterozygous in the low-SCE LCLs and regions distal to BLM that had become

homozygous. The minimal region to which BLM was thus assigned by SCP mapping is

represented in black.

Please amend the paragraph on page 4, line 19 through page 5, line 11 as follows.
Note that the underlining of "Clin. Genet." and "Am. J. Hum. Genet." appears in the
original text.

Figure 1B represents the autoradiographic evidence showing heterozygosity

proximal to BLM and reduction to homozygosity distal to BLM. The four persons of five

from whom low-SCE LCLs had been established that were informative at D15S1108 or

D15S127 are shown. To determine both the constitutional and the recombinant cell line

genotypes, PCRs were carried out using DNA samples prepared from high-SCE cells (P^h)

and low-SCE LCLs (P^l) of persons with BS as well as samples from their fathers (PF) and

their mothers (PM). These persons are identified by their Bloom's Syndrome Registry

designations (see German, J., and Passarge, E. Clin. Genet. 35:57-69 (1989)). Arrows

point to DNA fragments amplified from the heterozygous alleles of the constitutional

genotypes, pat (for paternal) and mat (for maternal). Asterisks mark alleles in the low-

SCE LCLs that are lost through somatic crossing-over. Lines mark DNA fragments

amplified from alleles of the parents but that were not transmitted to the offspring with

BS. From one of the four persons with BS, 11 different clonal LCLs were examined; 3 of

the 11 had undergone reduction to homozygosity at loci distal to BLM--as explained

elsewhere ((Ellis, N. A., et al. Somatic intragenic recombination within the mutated locus

BLM can correct the high sister-chromatin exchange ~~high-SCE~~ phenotype of Bloom

syndrome cells. Am. J. Hum. Genet. 57(5): 1019-27, 1995, Erratum in Am. J. Hum.

Genet. 58(1): 254, 1996 ~~in press~~). Autoradiographic patterns are shown from 2 of the 11

low-SCE LCLs from 11(IaTh), one representative of cell lines in which allele losses were

detected (P^1 sample on right) and another of cell lines in which they were not (P^1 sample

on left).

E5

Please amend the paragraph on page 5, lines 12-30 as follows. Note that the

KK 9/24/03 underlining of "J. Cell Biol." appears in the original text.

Figures 2A-2D ~~Figure 2~~ is *are* depictive of the 4,437-bp H1-5' sequence (SEQ ID NO:72), which

represents the merged sequences of the H1 cDNA and the 5' clones, with its encoded

1,417-residue amino acid sequence (single-letter code) (SEQ ID NO:78). Nucleotides in

the open reading frame starting at the first in-frame ATG, 75 bp from the first nucleotide

of the H1-5' sequence, are capitalized. The in-frame nonsense codon (TAA) marked by a

period is followed by 88 nucleotides of 3' untranslated sequence. At the initiator

methionine, there is a Kozak consensus sequence (Kozak, M. J. Cell Biol. 108:229-241

(1989)), and an acceptable polyadenylation sequence (underlined) is present 20-bp

upstream of a 21-bp polyA tail. Sites at which substitution or deletion were detected in

persons with BS (see Table 1) are boxed, and a site at which an insertion was identified is

marked by a diamond. The EagI and SmaI sites used in the construction of a full-length

cDNA referred to as B3 (see Experimental Details Section) are overlined. Asterisks mark

amino acid identities to three motifs present in the RNA polymerase II largest subunit.

E6

Please amend the paragraph on page 5, line 31 through page 6, line 12 as follows:

Figure 3 is depictive of the nucleotide sequence of the 5' end of the candidate gene

determined by cDNA analysis and 5'-RACE experiments. The sequence of the longest

E7

cDNA isolated (clone R1) is shown (SEQ ID NO:73). The sequences were obtained by analysis of 11 lymphoblastoid cDNAs (clone names prefixed by an R), identified by screening 8 X 10⁶ clones with a EagI/SmaI DNA fragment from the 5' part of the H1-5' sequences (FIG. 2), and of 12 5'-RACE clones amplified from fibroblast cDNA with nested PCR primers (Experimental Details Section). Vertical lines mark the nucleotides at which nine lymphoblastoid cDNA (clones named above the sequences) and six cloned 5'-RACE fragments (clones named below the sequences) initiated. Three cDNA and six 5'-RACE clones not shown contained sequences which initiated less than 38 bp upstream of the first in-frame ATG. The sequences at the 5' end are G+C-rich (71%), perhaps explaining the absence of in-frame nonsense codons upstream of the first in-frame ATG.

Please amend the paragraph on page 6, lines 13-30 as follows. Note that the underlining of "Nucl. Acids Res." appears in the original text.

Figure 4 represents the amino acid sequence homologies in the seven conserved helicase domains between the putative peptide encoded by the H1-5' sequence (BLM, SEQ ID NOS:74) and by the three other known members of the RecQ subfamily of helicases (REQL, SEQ ID NOS:75; SGS1, SEQ ID NOS:76; recQ, SEQ ID NOS:77). The numbers (left) indicate amino acid positions in each peptide, and gene product names are at the right. Sequence alignments were performed by the Megalign computer program (DNAStar); dashes indicate gaps inserted by the program to maintain alignment. Amino acid residues that are identical at a position between sequences are shaded. Two different shadings are used when at a position two pairs of identical amino acids were observed. Overlined sequences mark the seven helicase domains (Gorbalenya, A. E., et al. Nucl. Acids Res. 17:4713-4730 (1989)). The DEXH box is in helicase domain II. Asterisks denote positions at which putative missense mutations were identified. The candidate gene product is referred to here as BLM because mutations have been discovered in the

E8

gene in persons with BS (see text).

Please amend the paragraph on page 13, line 28 through page 14, line 16 as follows. Note that the underlining of "supra" and the Journal titles appear in the original text.

The persons with BS in whom low-SCE lymphocytes have arisen were described previously (German, J., et al. Bloom's syndrome. XIX. Cytogenetic and population evidence for genetic heterogeneity. Clin. Genet. 49(5):223-231, 1996 ~~, 1995, in press~~). Epstein-Barr virus transformed lymphoblastoid cell lines (LCLs) were developed from these and other persons with BS by standard culture methods using material obtained through the Bloom's Syndrome Registry (German and Passarge, supra). The recombinant low-SCE LCLs in which reduction to homozygosity had been detected, and the cells used to determine the constitutional genotypes of the five persons from whom these recombinant low-SCE LCLs were developed, also have been described (Ellis, et al. Am. J. Hum. Genet., 1995, supra). The polymorphic loci typed included some previously reported (Beckmann, J. S., et al. Hum. Mol. Genet. 2:2019-2030 (1993); Gyappay, G., et al. Nature Genetics 7:246-339 (1994)) and others that were identified during the physical mapping of the BLM region of chromosome 15 (Straughen, et al., supra). The methods of preparation of DNA samples, oligonucleotide primers, and conditions for PCR amplification of microsatellite polymorphisms on chromosome 15 have been described (German, et al., 1994, supra; Ellis, N. A., et al. Am. J. Hum. Genet. 55:453-460 (1994); Straughen, et al., supra).

Please amend the paragraph on page 14, line 19, through page 15, line 26, as follows. Note that the underlining of the Journal titles appear in the original text.

E10

Direct cDNA selection was carried out as described by Parimoo, S., et al. (Proc. Natl. Acad. Sci. USA 88:9623-9627 (1991)). Briefly, DNAs (15 ng) from commercial

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lambda cDNA libraries prepared from cultured foreskin fibroblasts (Clontech) and Jurkat cells (Stratagene) were amplified by PCR (94°C 1 min, 55°C 1 min, 72°C 2 min and 10 sec for 32 cycles) using primer set A (GGTGGCGACGACTCCTGGA (SEQ ID NO:1) and ACCAGACCAACTGGTAATG (SEQ ID NO:2)) for the fibroblast cDNA library and the universal forward and reverse M13 sequencing primers for the Jurkat cDNA library under standard conditions with Tag polymerase (Boehringer Mannheim). EcoRI-digested cosmid (c905) or P1 (P1958) DNAs (100 ng) bound to Hybond N membrane in 10 X SSC, were denatured in 0.5 M NaOH/1.5 M NaCl, neutralized in 0.5 M Tris-HC1 pH 7.2/1.5 M NaCl, and fixed by UV-crosslinking. Hybridization of the PCR-amplified cDNAs to repetitive sequences on the cosmid and P1 clones was blocked by prehybridizing the membranes with Cot1 DNA (25 ng/m; Gibco, BRL), poly(dI):poly(dC) (20 ng/μl; Pharmacia), vector DNA (pWE15 or pAD10SacBII at 25 ng/μl in 5 X SSPE, 5 X Denhardt's solution, and 0.5% SDS at 65°C overnight. Hybridization of the PCR-amplified cDNAs (25 ng/μl) was at 65°C for 2 days in the same solution without poly(dI):poly(dC). The membranes were washed, and without elution the bound cDNAs were amplified by PCR with primer set A, followed by nested PCR with primer set B (ATGGTAGCGACCGGCGCTCA (SEQ ID NO:3) and CCGTCAGTATCGGCGGAATT (SEQ ID NO:4)) for the fibroblast library and the T3 and T7 sequencing primers for the Jurkat library. A sample of the PCR product after each amplification was analyzed by agarose gel electrophoresis, and another was cloned into Bluescript. Independent clones were picked at random, plasmid DNAs prepared, and insert sizes were determined by restriction enzyme digestion and agarose gel electrophoresis. Inserts from selected clones were purified and used as hybridization probes against all of the other clones as well as against selected genomic DNAs to determine the chromosomal origin of the sequences (see below). The enrichment procedure was repeated and the selected cDNA clones analyzed again. The fibroblast cDNA clone 905-28 was obtained after two rounds of selection (250,000-fold enriched), and was sequenced by the dideoxy chain-termination technique (Sanger, F., et al. Proc.

Natl. Acad. Sci. 74:55463-5467 (1977); Tabor, S., and Richardson, C. C. Proc. Natl. Acad. Sci. USA 84:4767-4771 (1987)).

Please amend the paragraph on page 16, line 22, through page 17, line 3, as follows. Note that the underlining of "J. Mol. Biol." appears in the original text.

E^{11}

Because the reading frame was open at the 5' end of the H1 clone, additional upstream sequences were obtained by a PCR method. PCR was carried out on DNA prepared from the HeLa cDNA library using an oligonucleotide (Y177, TTGTGGTGTTGGGTAGAGGTT) (SEQ ID NO:5) 8 bp from the 5' end of H1 and the T3 sequencing primer. The PCR products were cloned into pT7Blue (Novagen), 18 clones were isolated, and the 8 largest inserts were sequenced. The three largest of these clones (5'-5, 5'-15, and 5'-17) extended the sequences 289 bp 5' of the H1 cDNA. The complete cDNA sequences present in the HeLa library are referred herein as H1-5' (FIG. 2). Database searches then were carried out according to the method of Altschul, S. F., et al. (J. Mol. Biol. 215:403-410 (1990)) using segments of the predicted amino acid sequence encoded in the HI-5' sequence as queries against the collected amino acid sequence databases that are accessible through the National Library of Medicine.

Please amend the paragraph on page 17, lines 4-10, as follows:

E^{12}

A full-length clone referred to as B3 was constructed by performing PCR of HeLa library DNA using an oligonucleotide (Y180, GCCGCCGGCACCAAC) (SEQ ID NO:6) from the 5' end of the H1-5' sequence and an internal oligonucleotide (BC13, CCTCAGTCAAATCTATTTGCTC) (SEQ ID NO:7) which permitted amplification of a 739-bp product. EagI and SmaI sites (Fig. 2) were used to clone the product into NotI/SmaI-digested H1 DNA.

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Please amend the paragraph on page 17, line 20, through page 18, line 3, as follows:

5'-RACE (rapid amplification of cDNA ends) was performed to characterize the 5' sequences of the candidate gene using a Clontech Marathon™ cDNA Amplification Kit according the manufacturers specifications. Briefly, first-strand synthesis was carried out with MMLV reverse transcriptase using polyT-primed RNAs prepared from cultured fibroblast, lymphoblastoid, and HeLa cells and polyA+ RNA from placenta (provided in the kit). Then, second-strand synthesis was performed with RNAseH, *E. coli* PoLI, and *E. coli* DNA ligase. The DNA ends were made blunt with T7 DNA polymerase, and adapters with overhanging ends were ligated to the cDNA. Nested PCRs then were carried out using 5' oligonucleotides from the adaptor (AP1 and AP2) and internal 3' oligonucleotides from the H1-5' sequence (BC5, GCCATCACCGGAACAGAAGGAAA (SEQ ID NO:8); and BC11, TCTTCTGGAGAAGGTGGAACAA (SEQ ID NO:9)). Bands derived from the H1-5' sequences were identified in all four of the cDNA samples. PCR products from the 5'-RACE-amplified fibroblast cDNA was cloned into Bluescript, and the 5' ends of 12 clones were sequenced (Fig. 3).

E11

Please amend the paragraph on page 26, lines 11-30, as follows. Note that the underlining of "Science", "Hum. Mol. Genet.", and the first two occurrences of "Am. J. Hum. Genet." appears in the original text.

The problem of too little positional information in available families can be mitigated in exceptional situations in which linkage disequilibrium between the disease-associated gene and tightly-linked polymorphisms can be detected in a genetic isolate. In these cases localization of a gene to a short interval in the genome by haplotype analysis can be more exact than is possible using standard linkage analysis of family data (e.g., Kerem, B.-S., et al. <u>Science</u> 245:1073-1080 (1989); Sirugo, G., et al. <u>Am. J. Hum. Genet.</u>

E12

50:559-566 (1992); Lehesjoki, A. E., et al. Hum. Mol. Genet. 2:1229-1234 (1993);

Hastbacka, J., et al. Cell 78:1073-1087 (1994)). Linkage disequilibrium in fact was a

strategy available in BS (Ellis, et al., Am. J. Hum. Genet., 1994, supra), and it permitted a

minimum regional assignment of BLM to the same 250-kb interval described herein (Ellis,

N.A. et al., The Ashkenazic Jewish Bloom syndrome mutation blmAsh is present in non-

Jewish Americans of Spanish ancestry, Am. J. Hum. Genet. 63(6): 1685-93, 1998

~~Linkage-disequilibrium mapping permits assignment of the Bloom's syndrome gene BLM~~

~~to a 250-kb genomic DNA segment on chromosome 15. Genomics, submitted~~). This

approach could have allowed the inventors to clone BLM. Instead, the inventors carried

out SCP mapping first.

E12